AMENDMENTS TO THE SPECIFICATION

Please replace the existing title, with the following rewritten title:

--METHOD OF DETECTING A CANCEROUS CELL EXPRESSING EGFL6, AN EGF MOTIF PROTEIN--

Please replace the existing paragraph beginning at line 1 of the Abstract, with the following rewritten paragraph:

--The present invention provides novel polynucleotides and proteins encoded by such polynucleotides, along with therapeutic, diagnostic and research utilities for these polynucleotides and proteins. In particular, the polypeptides of the invention ~~is~~ are useful for detecting cancers.--

Please replace the existing paragraph beginning at page 1, line 4, with the following rewritten paragraph:

--This patent application is a continuation-in-part of U.S. patent application Serial No. 09/687,860 filed October 13, 2001 (pending) which is a continuation-in-part of U.S. Patent Application Serial No. 09/363,316 filed July 28, 1999, now U.S. Patent No. 6,392,019. All of these applications are herein incorporated by reference in their entirety.--

Please replace the existing three (3) paragraphs beginning at page 17, line 7, with the following rewritten three (3) paragraphs:

--FIG. 4 shows SEQ ID NO: 5 or 23, and the amino acid translation (SEQ ID NOS: 6 or 24). SEQ ID NO: 5 ~~which~~ is a ~~five~~-5-prime and ~~three~~-3-prime extension of the cDNA sequence, SEQ ID NO: 2. Resequencing of pEGFR-HY2 and pEGFR-HY3 indicated an error in SEQ ID NO: 5 as presented in Figure 4 and clarified an ambiguous nucleotide within the coding region. Nucleotide 244 was reported to be a cytosine (C) in SEQ ID NO: 5 and should be a thymidine (T). Nucleotide 1273 was reported to be a tryptophan (W) in SEQ ID NO: 5 and should be an adenine (A). The correct sequence is presented in SEQ ID NO: 23.

~~FIG. 5 shows~~ SEQ ID NO: 6 or 24 is the amino acid translation ~~(SEQ ID NO: 6 or 24)~~ from nucleotide 205 to 1866 of SEQ ID NO: 5 or 23, including the starting methionine and stop codon. The first 21 amino-acids comprise the hydrophobic region that represents the signal peptide. The sequencing error described above caused an error in the translated amino acid sequence shown in SEQ ID NO: 6 where a proline (P) residue was reported at amino acid 14. The corrected nucleotide

sequence (SEQ ID NO: 23) resulted in a serine (S) at position 14 and an isoleucine at position 357, and this corrected amino acid sequence is presented as SEQ ID NO: 24.

FIG. 5 6 shows three-dimensional ribbon diagrams comparing the peptidyl backbone of amino acids 221-260 of EGFL6 with that of the 53 amino acid EGF protein. Although amino acids 221-260 of EGFL6 show only 22% identity with the amino acid sequence of EGF, 5 out of the 6 cysteines in EGF are conserved and the tree-dimensional structures look similar to each other.--

Please replace the existing paragraph beginning on page 30, line 3 with the following rewritten paragraph:

--Following the methods of Example 1, below, a splice variant (SEQ ID NO: 27) and a SNP (SEQ ID NO: 29) of EGFL6 were identified. The predicted amino acid sequence of SEQ ID NOs: 27 and 29 are set forth in SEQ ID NOs: 28 and 30, respectively. Sequence analysis determined that SEQ ID NO: 27 has a 6 amino acid insertion at about amino acid 30 of SEQ ID NO: 23; and is 98% identical to the amino acid sequence of EGFL6. Sequence analysis also revealed that SEQ ID NO: 29 has a His residue inserted at about amino acid 29 of SEQ ID NO: 23, and is 99% identical to the amino acid sequence of EGFL6. An additional SNP, which was previously identified as SEQ ID NO: 189 in U.S. patent application 09/620,312 (now U.S. Patent No. 6,569,662) filed July 19, 2000, is set forth in SEQ ID NO: 31. The amino acid encoded by SEQ IDNO: 31 is set forth in SEQ ID NO: 32. Sequence analysis of this SNP shows that it contains an Ala residue inserted at about amino acid 395 of SEQ ID NO: 23.--

Please replace the existing paragraph beginning on page 96, line 1, with the following rewritten paragraph:

--Conditions for incubating a nucleic acid probe or antibody with a test sample will vary. Incubation conditions depend on the format employed in the assay, the detection methods employed, and the type and nature of the nucleic acid probe or antibody used in the assay. One skilled in the art will recognize that any one of the commonly available hybridization, amplification or immunological assay formats can readily be adapted to employ the nucleic acid probes or antibodies of the present invention. Examples of such assays can be found in Chard, T., An Introduction to Radioimmunoassay and Related Techniques, Elsevier Science Publishers, Amsterdam, The Netherlands (1986); Bullock, G.R. et al., Techniques in Immunocytochemistry, Academic Press, Orlando, FL Vol. 1 (1982), Vol. 2 (1983), Vol. 3 (1985); Tijssen, P., Practice and Theory of immunoassays: Laboratory Techniques in Biochemistry and Molecular Biology, Elsevier Science

Publishers, Amsterdam, The Netherlands (1985). The test ~~s~~samples of the present invention include cells, protein or membrane extracts of cells, or biological fluids such as sputum, blood, serum, plasma, or urine. The test samples used in the above-described method will vary based on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can be readily be adapted in order to obtain a sample which is compatible with the system utilized.--

Please replace the existing paragraph beginning on page 100, line 14, with the following rewritten paragraph:

--Another aspect of the subject invention is to provide for polypeptide-specific nucleic acid hybridization probes capable of hybridizing with naturally occurring nucleotide sequences. The hybridization probes of the subject invention may be derived from the nucleotide sequence of the SEQ ID NOS: 1, 2, 5, or 23. Because the corresponding gene is only expressed in a limited number of tissues, especially adult tissues, a hybridization probe derived from SEQ ID NOS: 1, 2, 5, or 23 can be used as an indicator of the presence of RNA of cell type of such a tissue ~~in a~~.--

Please replace the existing paragraph beginning on page 104, line 8, with the following rewritten paragraph:

-- A plurality of novel nucleic acids were obtained from cDNA libraries prepared from various human tissues including fetal skin, fetal liver spleen, and lung tumor, and in some cases genomic libraries derived from human chromosome, as described in Bonaldo et al., Genome Res. 6:791-806 (1996), using standard ~~per~~PCR, SBH sequence signature analysis and Sanger sequencing techniques. The inserts of the library were amplified with PCR using primers specific for vector sequences which flank the inserts. These ~~s~~samples were spotted onto nylon membranes and screened with oligonucleotide probes to give sequence signatures. The clones were clustered into groups of similar or identical sequences, and single representative clones were selected from each group for gel sequencing. In some cases the 5' sequence of the amplified inserts was then deduced using the reverse M13 sequencing primer in a typical Sanger sequencing protocol. PCR products were purified and subjected to ~~flourescent~~fluorescent dye terminator cycle sequencing. Single pass gel sequencing was done using a 377 Applied Biosystems (ABI) sequencer. ~~IN~~ In some cases, RACE was performed to further extend the sequence. Two (2) of these inserts from the b^2HFLS20W cDNA library prepared from human fetal liver-spleen tissue, as described in Bonaldo et

al., Genome Res. 6:791-806 (1996) have been identified as novel sequences not previously obtained from this library, and not previously reported in public databases. These sequences are shown in Figure 2 as SEQ ID NO. 1-2. The polypeptide sequence corresponding to the nucleic acid sequence of SEQ ID NO: 1 is shown in Figure 2 as SEQ ID NO: 3. The polypeptide sequence corresponding to SEQ ID NO: 2 is shown in Figure 2 as amino acid residues 1-502 of SEQ ID NO: 4 as the designation "NNN" in SEQ ID NO: 2 represents a sequence ambiguity. These amino acid sequences contain EGF motifs that have striking homology to the EGF motifs of Notch (from drosophila) and CD97.--

Please replace the existing paragraph beginning on page 106, line 25, with the following rewritten paragraph:

--To determine if SEQ ID No. 2 is expressed specifically in diseased or normal human tissues, a Northern blot analysis was performed. The entire cDNA insert was labeled with radioisotope using a multiprime labeling method. A high stringency wash was performed to ensure specific hybridization. The resultant hybridization pattern produced a total of five different bands (approximately 6.5, 4.0, 2.1, 0.5 and 0.2 kb). Two of these bands (6.5 and 4 kb) were *uniquely* expressed in a sample derived from a brain tumor (astrocytoma of cerebellum) and not in a panel of ~~5~~ samples from 19 other tissues (normal brain, kidney tumor and normal kidney, liver tumor and normal liver, lung tumor and normal lung, normal heart, pancreas, spleen and skeletal muscle and fetal brain, liver, lung, and skeletal muscle). The other 3 bands were expressed to varying degrees in the other tissues. These results indicate that the two higher molecular weight bands are specific to the brain tumor tissue and not to the other ~~5~~ samples surveyed. As EGF-motif containing receptors have been previously been linked to the progression of various cancers, we believe that the full length message to SEQ ID No. 2 is involved in brain tumor development.--

Please replace the existing paragraph beginning on page 110, line 14, with the following rewritten paragraph:

--SEQ ID NO: 6 (Figure ~~5~~4) is the amino-acid translation from nucleotide 205 to 1866 of SEQ ID NO: 5, including the starting methionine and stop codon. The first 21 amino-acids comprise the hydrophobic region that represents the signal peptide. EGF motifs are located at amino acid residues 80-93 of SEQ ID NO:6 (EGF motif 1), amino acid residues 95-128 of SEQ ID NO:6 (EGF

motif 2), amino acid residues 133-168 of SEQ ID NO:6 (EGF motif 3), amino acid residues 175-214 of SEQ ID NO:6 (EGF motif 4), amino acid residues 220-259 (EGF motif 5). A hydrophobic region suggestive of a possible transmembrane domain is located at amino acid residues 446-465 of SEQ ID NO:6, two potential N-glycosylation sites at amino acid residues 247 and 346 of SEQ ID NOS: 6 or 24, a potential tyrosine phosphorylation site at amino acid residue 509 of SEQ ID NOS: 6 or 24, and a RGD motif at amino acid residues 363-365 of SEQ ID NO:6.--

Please replace the existing paragraph beginning on page 111, line 1, with the following rewritten paragraph:

--Further analysis of the deduced amino acid sequence of SEQ ID NOS: 6 or 24 using SignalP prediction [Nielsen et al., Protein Eng., 10:1-6 (1997)] indicates that an 18-amino acid putative signal peptide region is located at the N-terminus. Further analysis of the hydrophobic portions at the C-terminus by the TMHMM server (~~http://genome.cbs.dtu.dk/services/TMHMM-1.0/~~Center for Biological Sequence Analysis, Technical University of Denmark, Lyngby, Denmark) indicate that these portions do not encode typical transmembrane domains. The presence of a signal peptide and the absence of a typical transmembrane domain suggests that this protein actually is secreted.--

Please replace the existing six (6) paragraphs beginning on page 116, line 17, with the following six (6) rewritten paragraphs:

--Fourteen prostate ~~adenocarcimona~~ adenocarcinoma tissue ~~s~~ samples of various ~~grade~~ grades and ~~stage~~ stages were collected from different patients and analyzed with 3 ~~s~~ samples of normal prostate tissue. Strong EGFL6 transcript expression was detected in 100% of the prostate adenocarcinoma tissues. Expression was detected in low Gleason Grade ~~s~~samples. EGFL6 expression was not detected in any normal prostate ~~s~~ sample tested.

Sixteen colorectal carcinoma tissue ~~s~~ samples of various grades were collected from different patients and analyzed with 3 ~~s~~ samples of normal colon tissue. 81.25% of the colon carcinoma tissues were positive for EGFL6 expression including the low grade ~~s~~samples. EGFL6 transcript expression was not detected in any normal colon ~~s~~ sample tested. In addition, EGFL6 transcript expression was detected in the plasma cells, lymphocytes and endothelial cells in the lamina propria and in the transition areas where dysplasia and carcinoma were closest.

Four melanoma tissue ~~s~~ samples of various stages and sites of origin were collected from different patients and analyzed with one sample of normal skin tissue. 75% of the melanoma tissues were positive for EGFL6 transcript expression including the low stage ssamples. EGFL6 transcript expression was not ~~detaetable~~detectable in the normal skin tissue ~~s~~ samples tested.

Three lymphoma ~~s~~ samples of various stages collected from different patients were analyzed with one sample of normal lymph node tissue. 66.7% of the lymphoma tissue ~~s~~ samples were positive for EGFL6 transcript expression. EGFL6 transcript expression was not detectable in normal lymph node tissue tested.

Three sarcoma ~~s~~ samples of various stages collected from different patients were analyzed with one sample of normal skeletal and smooth muscle tissue. 33.3% of the sarcoma tissues were positive for EGFL6 expression. EGFL6 expression was not detectable in normal skeleton and smooth muscle samples tested.

Three brain tumor ~~s~~ samples of various types were collected from different patients and analyzed along with one sample of normal brain tissue. 66.7% of the brain tumor ~~s~~ samples (menigioma and astrocytoma) were positive for EGFL6 transcript expression. EGFL6 transcript expression was not detectable in the sample normal brain tissue tested.--

Please replace the existing two (2) paragraphs beginning at page 117, line 15, with the following two (2) rewritten paragraphs:

--EGFL6 mRNA was detected in colorectal cancer tissues (see Examples 6 and 8) and therefore it was of interest to determine if EGFL6 protein correlates with EGFL6 transcript expression in colorectal cancer tissue. The immunohistochemical analysis was carried out as follows by QualTeck Molecular Laboratories (Santa Barbara, CA) with a polyclonal anti-human EGFL6 antibody directed against the EGFL6 peptide QDREDDFDWNPADR (SEQ ID NO: 33). The tissues used for the immunohistochemistry were obtained from the same paraffin blocks as those ~~s~~ samples in Example 8 but the sections were cut from a different level of the cut sections and therefore the tissue s were similar but not identical.

Fourteen colorectal cancer ~~s~~ samples of various ~~grade~~ grades and ~~stage~~ stages and three normal colorectal tissues were fixed in 10% neutral buffer formalin, paraffin-embedded and cut into 4 μm thick s ctions. Tissue sections were deparrifrinized by 4 immersions in xylenes followed by ~~a~~ an immersion in a graded alcohol series to distilled water.--

Please replace the existing paragraph beginning at page 119, line 1, with the following rewritten paragraph:

--Fourteen ~~s~~samples of colorectal carcinoma tissues of various grades were collected from different patients and analyzed with 3 ~~s~~samples of normal colon tissue. 71% of the colon carcinoma tissues were positive for EGFL6 protein expression, including the low grade ~~s~~samples, and expression was mainly localized in the cytoplasm. EGFL6 protein and mRNA expression was not detectable in any of the normal colon ~~s~~samples. Three of the tumor ~~s~~samples negative for EGFL6 protein expression were found to express EGFL6 mRNA in Example 8. One tumor positive for EGFL6 protein expression was found negative for EGFL6 mRNA expression in Example 8. Lymphocytes in the lamina propia were positive for EGFL6 protein expression similar to EGFL6 transcript expression. This immunohistochemical analysis demonstrates EGFL6 protein expression correlates with the detected EGFL6 transcript expression in colon carcinoma.--

Please replace the existing paragraph beginning at page 120, line 4, with the following rewritten paragraph:

--EGFL6-Fc cDNA was stably transfected into Cos-7 cells for recombinant expression. Initially, Cos-7 cells were seeded into 25 dishes (150 mm3) at 4 x 10^6 cells /dish. On the following day, the cells were transfected with the Fugene-6 reagent (Roche Molecular Biochemical) according to the manufacturer's instructions. For each dish, 100 µl of Fugene-6 and 30 µg of EGFL6-Fc cDNA were added to 2 ml of DMEM media and incubated at room temperature for 20 minutes. Subsequently, the 2 ml of DNA-Fugene mixture was added dropwise to each dish and the cells were incubated at 37°C in a 5% CO_2 incubator for 24 hours. The cells were then washed with PBS and fresh Hyclone PF-CHO serum free medium was added. After a 72 hour incubation at 37°C in a 5% CO_2 incubator, the conditioned medium was pooled, centrifuged and the supernatant collected.--